6

[*From the Journal of Physiology*, Vol. III., No. 3.]

6

NOTES ON THE TEMPERATURE OF HEAT-COAGULATION OF CERTAIN OF THE PROTEID SUBSTANCES OF THE BLOOD.

By E. A. SCHÄFER, F.R.S.

I.

If blood-serum be saturated completely with sulphate of magnesia, the whole of the serum-globulin which it contains is precipitated, and may be separated by filtration[1]. The only proteid that is known to be present in any appreciable amount in the clear filtrate is serum-albumin. This substance it is well known coagulates when in solution at temperatures varying between 60° C. and 75° C., the coagulation at the lower temperatures (60° or thereabouts) being incomplete, and sufficient only to render the fluid opalescent. The variation is largely due moreover to varying reaction of the fluid, the more completely neutral, the lower the temperature of heat-coagulation ; the more alkaline, the higher. It is to be expected therefore that the serum-albumin in the clear filtrate above mentioned will, if gradually heated, begin to coagulate at a temperature not lower than about 60° C., the complete coagulation of the albumin not occurring until a higher temperature is reached.

It is stated however by Fredericq[2], who seems first to have performed the experiment in the manner above indicated, that the serum-albumin (or serine) begins under the circumstances in question to become precipitated at a far lower temperature than would have been anticipated. Fredericq found that in serum saturated with magnesic sulphate and filtered, a considerable deposit frequently occurred at or a little above 40° C., and that as the temperature was gradually raised, fresh deposits occurred, until by the time 50° C. was reached, nearly the whole of the serine had been precipitated .

---

[1] Hammarsten. "Ueber das Paraglobulin." Pflüger's *Archiv*, XVII., 1878.

[2] Fredericq. "Recherches sur les substances albuminoides du sérum sanguin." *Arch. de Biologie*, Vol. I., 1880.

[3] Gulliver (Hewson's *Works*, p. 28, note XVII.) found that the heat-coagulation point of horse serum was lowered by the addition of an excess of sulphate of soda, but not to anything like the temperatures mentioned by Fredericq; not, indeed, falling below 144° F. (62° C.).

In rehearsing the above experiment for demonstration to a class last winter, I obtained, as I at first thought, a result which seemed to coincide with that of Fredericq. The serum employed was from the horse, and it had been saturated with magnesic sulphate by gradual addition of the salt and thorough shaking of the vessel with the hand. Two portions of the clear filtrate, which was slightly alkaline, were taken, and one of them was immersed in water, and subjected to a gradually increasing temperature, which was constantly noted by aid of a thermometer dipping into the liquid, while the other portion was at once plunged into a water bath, which had been previously heated to 43° C. In the latter an immediate and apparently abundant flocculent precipitate was produced ; in the former, a slight turbidity was noticed at as low a temperature as 35°, and gradually increased to a flocculent precipitate as the temperature was raised to 40°. Both portions of liquid were then allowed to cool, and were filtered, and the filtrate having been again gradually heated remained clear until 70° C. was reached. The first precipitate, which when collected was found to be only small in amount, was collected and found to dissolve in water with the aid of the adhering salts. Its solution was however precipitated by saturation with sulphate of magnesia. The precipitate obtained was therefore not serum-albumin, but serum-globulin ; and this latter had not been entirely thrown down from the liquid employed.

It is not to be supposed from this that Hammarsten's statement as to the complete precipitation of serum-globulin by saturation of its solution with sulphate of magnesia is incorrect. On the contrary, the next experiment which I made proved the correctness of it. For suspecting that the mere shaking of the vessel with the hand had not been sufficient to produce complete saturation, I added a quantity of the salt to what remained of the original filtrate, and placing it in a stoppered bottle, which it completely filled, I attached the bottle to an eccentric connected with a steam engine in the Engineering Laboratory, and in this way had the fluid shaken up with the salt for at least three hours. Examined at the end of that time it was decidedly turbid, and when a sample was filtered, and then gradually heated in the same way as before, no precipitate was produced until 65° C. was reached. At this temperature the previously clear fluid began to be cloudy : at 70° C. it was an almost solid white mass of coagulated albumin.

Similar results to the last were obtained with the serum of the blood of other animals (ox, sheep, pig, cat). In all cases it was found that if care be taken to saturate the serum as completely as possible—and if it be used in any quantity this can only be done by some such means as that which I employed[1]—coagulation was rarely obtained at any temperature below 60°, and never at the low temperature mentioned by Fredericq, even if the saturated and filtered serum were previously most carefully neutralized with dilute acetic acid.

Fredericq's result, viz., the precipitation at a low temperature in fluids which, as I think, had not been completely saturated with the salt, was best marked with horse serum (which he seems to have chiefly employed). With a sample of ox serum saturated by agitation of the vessel by the hand only, a very slight flocculent deposit occurred at 55° C.; but when this was filtered off, and the temperature was again raised, no more precipitation appeared until a temperature of about 75° C. was reached. Another sample of the same saturated and filtered serum which was similarly treated the next day gave no flocculent deposit at the lower temperature, but was precipitated, as before, at about 75° C.; and another, which had been carefully neutralized, and was kept for some minutes at 56° C., also gave no deposit. The coagulation temperature in the latter was not further examined. The flocculent deposit which was obtained in the imperfectly saturated fluids was always soluble in water by aid of the adhering salts; the white coagulum produced at the higher temperatures was, it is scarcely necessary to state, completely insoluble.

It would appear, therefore, that serum-globulin is less soluble in warm serum nearly saturated with $MgSO_4$ than in the same solution in the cold. To test if this were the case with a purer solution, I took some serum-globulin (obtained by precipitation from horse's blood and purified by redissolving in water and reprecipitating it by $MgSO_4$ three times), and having dissolved it in weak solution of $MgSO_4$, added that salt to almost complete saturation (effecting the saturation as before by agitation with the hand). On filtering, and slowly warming some of the filtrate, a slight opalescence occurred at 56°. Another portion of the filtrate was then completely saturated with $MgSO_4$; a slight additional deposit became thereby produced corresponding, apparently, to the amount of precipitate produced in the former sample. The result of this experiment was not conclusive, for the temperature at which the deposit occurred was distinctly higher than that at which it usually occurs in incompletely saturated serum.

---

[1] Hammarsten also insists upon the difficulty of completely saturating serum with sulphate of magnesia, and upon the importance of arriving at complete saturation in order to obtain complete precipitation of the globulin.

II.

Blood-serum, which had been completely saturated with $MgSO_4$ and filtered from precipitated globulins, was then saturated with Na_2SO_4. The dense, sticky precipitate obtained was collected on a filter, and washed with water which had been saturated with both salts. The precipitate (serine) dissolved readily in water, some of the salts remaining adherent to it, and its solutions were not precipitated by saturation either with $MgSO_4$ or with Na_2SO_4 alone, although they were completely precipitated by saturation with both those salts. By re-solution and re-precipitation it can be purified to a great extent. Its solutions are coagulated at about 70° or upwards, but I have not determined the coagulation point with sufficient exactness with reference to the amount of salt present and the animal from which the serum has been taken—circumstances which probably cause considerable variation in the point of heat coagulation. Its solutions are not precipitated by prolonged dialysis.

After the removal by filtration of the above second precipitate (of serine, the first being the serum-globulin precipitated by saturation with $MgSO_4$ alone), the filtrate which collected below was a beautiful crystal-clear fluid, of a bright yellow tint in serum from the horse, but perfectly colourless in serum from the cat. Some of this filtrate from serum of the cat was taken and heated gradually. It remained perfectly clear until a temperature of 85° C. was reached, when an opalescence was produced, gradually increasing as the temperature was raised ; but it was only after boiling the fluid for a minute that a distinct coagulation was produced sufficient to cause the fluid to become thick.

Another sample was subjected to prolonged dialysis in a sausage-tube dialyzer placed in a current of water, at first ordinary water but afterwards distilled water being used. No precipitate was thereby produced. After testing the fluid and finding that only the merest trace of sulphates remained, it was as before subjected to a gradually increasing temperature. Now that the salts were removed it was found that the fluid might be boiled without the production of any precipitate. It may therefore be concluded that the proteid-residue which is left in serum after the precipitation of serine by the double saturation with $MgSO_4$ and Na_2SO_4 is albumin, but it is apparently not the same as the ordinary serum-albumin or serine. For it differs from this by its non-precipitability by the double saturation, or in other words in being soluble in the double saturated solution ($MgSO_4$ and Na_2SO_4) and, perhaps, in having a higher heat-coagulation point, although from experiments made under such varying circumstances this cannot be positively affirmed.

With regard to the amount of this proteid-residue all that can be at present stated is, that it is much less than that of the serine, and forms but a small proportion of the total proteids.

III.

On the Temperature of Heat-Coagulation of Fibrinogen.

The remarks that I have to offer on this point are chiefly historical, and are made with the view of urging a claim of priority for English physiologists. The important fact that the temperature of heat-coagulation of fibrinogen is as low as 56° C., so that by heating a fibrinogenous liquid to this point the whole of its fibrinogen is precipitated and its ferment-coagulability permanently destroyed, is illustrated by the following interesting experiment which is generally ascribed to Fredericq:—If the jugular vein of a horse be tied below and above, and then carefully excised from the living or recently killed animal, it is well known that the included blood will remain uncoagulated for a considerable time, since it is in contact merely with the uninjured inner surface of the vein. If now the vein with its contents be heated to 56° a proteid substance (fibrinogen) separates out, and the blood is thereby rendered uncoagulable by ferment[1].

This experiment is not, however, due to Fredericq, although he has the merit of its rediscovery, and with the aid of the facts which have been elucidated by modern research, has been able to point out its true significance. It was performed by Hewson in almost exactly the same manner, as will be seen from the following extract (*Works*, edited by Gulliver, p. 26): "Besides being coagulated when exposed to the air, the coagulable lymph, as well as the serum, is known to be fixed by heat; but the degree of heat has not, so far as I know, been determined. It has been supposed to require a degree almost equal to that which coagulates the serum, but one much less is necessary, as will appear from the following experiments:—Experiment VII. Having found, from a number of trials, that blood kept fluid by means of true Glauber's salt had its lymph coagulated by a heat of 125° of Fahrenheit's thermometer (nearly 52° C.), I supposed that the degree necessary for fixing it in its natural state could not be very different from this. I therefore prepared a lamp furnace with a small vessel of water upon it: this water was heated to 125°; and then laying bare the jugular vein of a living dog, I tied it properly, cut a piece of it out, and put it into this water; after eleven minutes, I took out the vein, opened it, and found the blood entirely coagulated: thence I concluded that 125° or less was sufficient to coagulate the blood of a dog. It may be necessary to observe here that the part coagulated was only the lymph; for the serum requires a much greater heat to fix it, that is, a heat of 160°."

---

[1] Fredericq. *Recherches sur la constitution du plasma sanguin.* Gand, 1878.

Mr. Gulliver makes the following remark on the above experiment, having himself repeated and confirmed it with blood and liquor sanguinis kept fluid by salt:—" It is true that in salted liquor sanguinis at 124°—127°, though a great part of the mixture remains fluid, a little curdy, flakey, filamentous and opaque precipitate forms, which is probably what Hewson describes in his Experiment VII. as coagulation; though it differs from the spontaneous setting or jellying into a transparent mass of a mixture of salted liquor sanguinis and water" (p. 5, note III., *f*).

In narrating the repetition of Hewson's experiments, Mr. Gulliver is very explicit. Speaking of a mixture of liquor sanguinis and salt diluted with distilled water, he says:—" From 124° to 127° its power of spontaneous coagulability is completely destroyed" (p. 6, note *h*). Further:—" Some liquor sanguinis skimmed from fluid blood, and kept at a heat of 139° (about 59·5 C.) for twenty minutes never coagulated" (p. 5, note *c*).

It will be seen, therefore, that the temperature of heat-coagulation of fibrinogen (or the coagulable lymph, as it was then termed) was carefully ascertained by Hewson, both for salted blood and blood kept fluid in a living vein; and the temperature which he obtained (nearly 52° C.) agrees with the lowest estimate of Hammarsten[1]. On the other hand, Hewson does not, in his description at least, distinguish between the coagulation produced by heat and that occurring spontaneously, although it is probable that the difference of appearance did not escape so acute an observer. But it must be remembered that the important difference between the two processes to which the name of coagulation is given, and which we can now distinguish as heat-coagulation and ferment-coagulation, was not then recognized, and the same word was used indiscriminately for both. Gulliver, on the other hand, arrived much more closely at the understanding of the change produced in liquor sanguinis by a comparatively low temperature, affirming most positively the destruction of its coagulability, accompanied by an albuminous (proteid) deposit, at temperatures between 124 F. and 139° F. (51° C. and 59° C.). Had these experiments of Hewson and Gulliver been repeated and followed up it is probable we should not have had to wait until the present time to have arrived at a better knowledge of the substance which was precipitated in their experiments, and which is now known as fibrinogen.

---

[1] In one experiment Hewson obtained heat-coagulation at a temperature of 120·5° F., but not below this. The difference may be due to the source from which the blood is taken.

In conclusion, I may state that I have two or three times had the opportunity of repeating the above experiment of Hewson and Fredericq upon the jugular vein (of the horse) with a result perfectly similar to that which is described by the last-named observer in his able essay upon the subject. For purposes of demonstration, however, I would suggest a modification which renders the result more striking:—Having excised the tied vein, let it be suspended for two or three hours in a cool, moist atmosphere by the ligature which is attached to its anterior end. By this time there will be a considerable stratum of clear plasma at the upper part of the suspended vein. Some of this is now drawn off into a pipette, and, without a moment's delay, transferred to a test tube, which is immersed in a large beaker of water heated to 56·5° C. A second portion is removed in the same way and placed in a test tube which is exposed to the ordinary atmospheric temperature. In the first test tube a sticky, flocculent precipitate of fibrinogen appears, and when this is filtered off the fluid is found to have lost its ferment-coagulability. In the second test tube the plasma speedily sets into a clear, or but slightly turbid, gelatinous clot.